CLL Chromosome and Gene Abnormality Detection Kit （FISH）
Instruction For Use

[Product Name]

CLL Chromosome and Gene Abnormality Detection Kit (FISH)

[Specification]

5 tests/kit, 10 tests/kit, 20 tests/kit.

[Intended Use]

This reagent is used for immunohistochemical staining based on routine staining(such as HE stain) to provide auxiliary information for physicians in diagnosis.

Chronic lymphocytic leukemia (CLL) is a neoplastic disease characterized by clonal proliferation of lymphocytes, which accumulate in bone marrow, lymph nodes, blood, spleen, liver and other organs. More than 95% CLL are clonal proliferations of B cells (B-CLL), and less than 5% of cases have a T-cell phenotype (T-CLL). Recent clinical findings show that the occurrence and development of CLL is accompanied by various genetic factors and chromosomal abnormalities. For example, nearly 50% of CLL patients have deletions in the long arm of chromosome 13. The deletion site can affect the tumor suppressor gene RB-1 (retinoblastoma gene). The deletion of the short arm of chromosome 17 is only found in 10% to 15% CLL patients. These patients have p53 gene mutations. FISH technology is the earliest clinical test method for leukemia analysis. It uses various known genes related to blood diseases as detection targets, analyzes and judges condition and cause of patients by the different expressions of such genes or special segments on cell chromosomes. This can be used as a basis to assist in the decision of subsequent treatment plans or to monitor the prognosis of patients.

[Test Principle]

Based on the principle of complementary base pairing, marked the fluorescent stain in DNA fragments (probe) matching with the target DNA.The probe and the corresponding DNA fragments in material waiting for test specificaly combined under certain conditions (hybrid), to form double-stranded nucleic acid, by means of fluorescence microscope to observe and record the hybrid double chain type, quantity, to judge target DNA in inspected samples normal or not.

The samples were fixed by 10% neutral formalin, paraffin-embedded and sliced, then placed on a slide. The denatured DNA formed a single strand state and hybridized with the probe. After hybridization, unbound probes are washed away. Use DAPI (4,6- diimiyl -2- diphenylindoles) to restain the nucleus. DAPI is a nucleic acid stain used as a specific DNA stain. The kit’s probe hybridization signals can be observed by fluorescence microscopy equipped with corresponding

filters (blue, red and green fluorescent signals).Fluorescence signals in the nucleus were observed under a microscope.

[Main Components]

The kit is mainly composed of p53/CEP17 probe, ATM/RB1 probe, DLEU/Cep12 probe and DAPI counterstain solution.

The reagent does not contain but necessary for the test: Blood (bone marrow) cells Sample Pretreatment Kit (FISH).

Other reagents not provided: Xylene; Ethanol (anhydrous, 85%, 70%); methanol; glacial acetic acid; deionized water; Sealing gum.

[Storage and Validity]

Stored at -20℃±3℃ in dark, sealed conditions, valid for 12 months.

Shipping conditions: The shipping temperature should not exceed 8℃, and the time should not exceed one week.

[Applicable Instrument]

Fluorescence in situ hybridization system;

Fluorescence microscope, in which:

Objective lens: It is recommended to use 100 times achromatic objective lens of immersion type, which can obtain satisfactory results.

Immersion Oil: Low level self-fluorescence Immersion Oil exclusive use for fluorescence microscopy.

Optical Filters: It is recommended to select filters suitable for labeled fluorescent stains.

Green fluorescence: excitation wavelength is 490nm , and emission wavelength was 516nm.

Orangered fluorescence: excitation wavelength was 551nm ,and emission wavelength was 572nm.

[Sample Requirements]

Collect specimens: bone marrow (2-3ml) or peripheral blood (3-5ml). Heparin sodium anticoagulation. The amount of specimens depends on the specific conditions of the patient such as aplastic anemia patients with less leukocytes should increase the collection.

Specimen storage and transportation: after collection, it should be processed within 2h. Stored at 4℃ ~ 8℃.

[Test Method]

1. Testing instruments and equipment: in situ hybridization system, fluorescence microscope, centrifuge, water bath.

2. Reagent preparation

2.1 Pepsin digestion solution: Add 1 tube pepsin powder (0.1g) per 50 mL pepsin solution. Placed in a 37±1℃ water bath to keep warm for later use.

2.2 Stationary liquid

The cell stationary liquid was prepared according to the ratio of methanol (AR): glacial acetic acid (AR) = 3:1. The stationary liquid is fresh prepared and used immediately.

3. Cell collection and slide preparation

3.1 Take out 2~3ml peripheral blood or bone marrow, centrifuge and discard the supernatant.

3.2 Add 10 mL hypotonic buffer, put in the constant temperature water bath at 37℃ for 20 minutes

3.3 Add 1ml stationary liquid, and fix for 10min at room temperature. Centrifuge at 2000rpm for 10 minutes, discard the supernatant

3.4 Add 10ml stationary liquid to resuspend, centrifuge at 2000rpm for 10 minutes, discard the supernatant. Repeat three times.

3.5 Prepared single cell suspension solution, conventional drop slides, aging for 30min;

4. Slide treatment

4.1 Add preheated pepsin digestion solution, digest for 2-10min

4.2 Rinse slides twice with purified water, 5 min/time

4.3 Remove the slide, the slides were successively treated in 70%, 90%, and anhydrous ethanol solution for 2 min. Remove the slide, dried at room temperature.

5. Denaturation and hybridization (avoid light)

5.1 Remove the probe,mix and centrifuge. Add 10ul probe and drop it on the target area. Cover the coverslip and avoid air bubbles.

5.2 Seal it with the sealing gum. Note that the coverslips are completely prevented from volatilizing the hybridization solution.

5.3 Put the slides into a hybridization instrument, denature for 6min at 83℃, denature and hybridize at 40℃ overnight.

6. Washing after hybridization

6.1 30 minutes before washing. Put the washing buffer I into the water bath and heat it to 72±1℃.

6.2 Gently remove the sealing gum from the slide with tweezers. Soak the glass slides in the washing buffer II solution, placed for 5 minutes, and remove the coverslip.

6.3 Put the slide into washing buffer I at 72±1℃, placed for 2 minutes

6.4 Remove the slide, soak in the washing buffer II solution at room temperature, placed for 2min

6.5 Remove the slide in 70% and 85% ethanol solution for 2min in turn

7. Add 10ul DAPI to the target area of the slide and cover with a coverslip. The slides were placed in -20℃ condition for 10-20 minutes and then taken out for observation and analysis under a fluorescence microscope.

[Positive judgment value]

For the P53/Cep17 combination, 200 cells were analyzed for each sample. There are two orange signals and two green hybridization signals in the normal case, and no green signal or only one green signal in the abnormal case. The proportion of abnormal cells greater than 3% is interpreted as positive, and less than 3% is negative.

For the ATM/RB1 combination, 200 cells were analyzed for each sample. There are two orange signals and two green hybridization signals in the normal case, and no orange signal or only one orange signal or no green signal or only one green signal in the abnormal case. The proportion of abnormal cells greater than 5% is interpreted as positive, and less than 5% is negative.

For the DLEU/Cep12 combination, 200 cells were analyzed for each sample. There are two orange signals and two green hybridization signals in the normal case, and three green signals in the abnormal case. There is chromosome 12 polysomy The proportion of abnormal cells greater than 8% is interpreted as positive, and less than 8% is negative.

[Attentions in result analysis]

1. Tumor cells should be randomly counted in the samples.
2. Counting cells must have clear and recognizable signals in each channel.
3. Do not analyze the heterogeneous regions.
4. Do not analyze nucleus with unclear outline or overlap.
5. Do not analyze the area where the background is so deep that affects the signal judgment.
6. Counting results shall be independently completed by two participants, and the results should be consistent before confirmation.
7. If the signal in over 25% of the nucleus is too weak, the region should not be analyzed.
8. If signals are present in more than 10% of the cytoplasm, the region should not be analyzed.
9. First judge the sufficiency of slide hybridization

Nuclear morphometry: The borders of the nucleus are usually clear and the nucleus is intact

Background state: The background should not contain particulate matter that affects the counts.

Fluorescence Signal Intensity: The signal should be bright, distinct and easy to count. The signal should exist in a bright, oval shape. Avoid overly scattered signals.

10. Fluorescence signal observation of the target area

Observe IGH signal with a 40× or 100× objective lens and appropriate filters. Adjust the size and shape of the focus to observe the target signal and noise (non-target hybridization signal). Make sure that the background does not have strong fluorescence interference signals.

Scan the whole slide to observe the overall fluorescence signal distribution of cells, and select a more representative area for counting.

11. Select and count cells within the target area

Select an area with better distribution of nuclei (such as areas that can distinguish single nuclei), and ensure that the selected area represents the observed signal distribution characteristics; Start analyzing and recording the fluorescence signal of each cell. Repeat the above operation until 200 cells are counted.

12. Signal counting rules

Adjust the depth of focus to locate the signal in the nucleus. Analyze the selected cells with sharp boundaries.

[Limitations of detection Methods]

1. This kit is an in vitro diagnostic reagent. The clinical evaluation of the test results should be combined with the patient's medical history and other clinical diagnosis results and should not be used as the sigle basis for clinical diagnosis and treatment.
2. This kit is only suitable for detection of P53, RB, ATM, DLEU abnormalities in CLL patients by fluorescence in situ hybridization, not for detection of other gene mutation modes.
3. If the test results are inconsistent with the histopathological characteristics, the pathological diagnosis should be verified or retested.
4. The performance of the product are obtained based on the test procedure described in the specification. Changes to this procedure may change the results .

[Product Performance]

1. Intensity of fluorescence signal

After hybridization of the probe with peripheral blood cultured lymphocytes and CLL tissue sections, fluorescence signals should be generated, and recognized by the naked eye under the fluorescence microscope.

2. Sensitivity

Choose 100 chromosomes 17 of 50 cells in metaphase phase were analyzed in each sample. At least 98 chromosomes showed 1 green fluorescence signal by 1 centromere region (17) and orange fluorescent signal marked by 1 gene locus region (P53). Choose 100 chromosomes 11 of 50 cells in metaphase phase were analyzed in each sample. At least 98 chromosomes showed 1 orange fluorescent signal marked by 1 gene locus region (ATM). Chromosomes 13, at least 98 chromosomes showed 1 green fluorescent signal marked by 1 gene locus region (RB1). Choose 100 chromosomes 12 of 50 cells in metaphase phase were analyzed in each sample. At least 98 chromosomes showed 1 green fluorescence signal by 1 centromere region (12) and orange fluorescent signal marked by 1 gene locus region (DLEU).

3. Specificity

For the P53/Cep17 combination, Choose 100 chromosomes 17 of 50 cells in metaphase phase were analyzed in each sample. At least 98 chromosomes showed their specific fluorescence signals in the centromere region and gene locus region, respectively. For the ATM/RB1 combination, Choose 100 chromosomes 11 and 13 of 50 cells in metaphase phase were analyzed in each sample. At least 98 chromosomes showed their specific fluorescence signals in the centromere region and gene locus region, respectively. For the DLEU/Cep12 combination, Choose 100 chromosomes 12 of 50 cells in metaphase phase were analyzed in each sample. At least 98 chromosomes showed their specific fluorescence signals in the centromere region and gene locus region, respectively.

[Cautions]

1. This kit is only used for in vitro diagnosis and must be used by strictly trained professionals.
2. In order to obtain ideal results, it is necessary to ensure that the reagents are correctly prepared and stored in accordance with the instructions.

[Symbols]

Symbol	Used for	Symbol	Used for
	Use-by date		Consult instructions for use
	Batch code		In vitro diagnostic medical device
	Temperature limit		Manufacturer
	Avoid overexposure to the sun		Date of manufacture

[Basic Information]

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